Developmental Biology **217,** 1–9 (2000)
doi:10.1006/dbio.1999.9493, available online at http://www.idealibrary.com on IDEAL®

REVIEW

Subdividing Cell Populations in the Developing Limbs of *Drosophila:* Do Wing Veins and Leg Segments Define Units of Growth Control?

Marco Milán and Stephen M. Cohen

European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

INTRODUCTION

The *Drosophila* limb primordia originate as groups of 20–40 cells in the embryonic ectoderm. The leg and wing primordia are specified as a single cluster of cells which splits into dorsal and ventral groups to form the leg and wing imaginal discs (reviewed in Cohen, 1993). During larval development the disc primordia grow rapidly and increase about one-thousand-fold in cell number. As the discs mature the pattern of the appendages is specified through localized expression of a number of transcription factors (reviewed in Neumann and Cohen, 1997b; Carroll, 1998). This ultimately leads to the production of a fine-grained pattern in which the number and position of structural features such as wing veins and sense organs are specified.

Over 25 years ago García-Bellido and colleagues recognized that the developing limb primordia are subdivided into developmentally separate domains, called compartments (García-Bellido *et al.*, 1973; reviewed in Blair, 1995; Lawrence and Struhl, 1996; Brook *et al.*, 1996). Operationally, compartments were defined in terms of the boundaries of cell lineage restriction that separate them. The anterior and posterior compartments derive from separate populations of founder cells in the embryonic ectoderm and the progeny of these founder cells do not mix during subsequent development. During early larval development a second lineage restriction boundary subdivides the wing disc into dorsal and ventral compartments (García-Bellido *et al.*, 1976). Subsequent work has shown that the compartments are defined by localized expression of transcription factors that specify compartment-specific cell fate: the homeodomain proteins Engrailed and Invected specify posterior fate and the LIM-homeodomain protein Apterous specifies dorsal fate (reviewed in Blair, 1995; Lawrence and Struhl, 1996; Brook *et al.*, 1996).

Soon after compartments were first identified it was recognized that the interface between these territories provided a discontinuity that could in principle serve as a source of information to pattern the discs (Crick and Lawrence, 1975). Short-range interaction between cells in adjacent compartments has subsequently been shown to lead to the expression of the secreted signaling proteins Wingless (Wg) and Decapentaplegic (Dpp) in cells adjacent to the compartment boundaries (Basler and Struhl, 1994; Tabata and Kornberg, 1994; Diaz-Benjumea *et al.*, 1994; Diaz-Benjumea and Cohen, 1995; Zecca *et al.*, 1995; Tabata *et al.*, 1995). Wg and Dpp form long-range activity gradients that further subdivide the imaginal discs into discrete regions by defining the domains of target gene expression along the anterior–posterior (AP), proximal–distal (PD), and dorsal–ventral (DV) axes of the limbs (Zecca *et al.*, 1996; de Celis *et al.*, 1996b; Nellen *et al.*, 1996; Lecuit *et al.*, 1996; Lecuit and Cohen, 1997; Brook and Cohen, 1996; Jiang and Struhl, 1996; Neumann and Cohen, 1997a,b; Sturtevant *et al.*, 1997; Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). The formation and maintenance of these secondary subdivisions does not use cell-lineage-based mechanisms like those used to form compartments (e.g., Brook and Cohen, 1996; Jiang and Struhl, 1996; Wu and Cohen 1999).

This review will address the formation of secondary subdivisions in the wing and leg. We will review recent literature on the subdivision of the wing into vein and intervein territories and subdivision of the leg into segments. We discuss some evidence which suggests that these subdivisions might function as semiautonomous units of pattern and growth control within the limbs.

PRIMARY AND SECONDARY SUBDIVISION ALONG THE AP AXIS OF THE WING

Primary Subdivision: Hedgehog Signaling and the AP Compartment Boundary

Compartment-specific expression of *engrailed* was proposed to produce a boundary of lineage restriction between anterior (A) and posterior (P) compartments by causing differential cell affinities between P and A cells (García-Bellido, 1975). Cells mutant for both *engrailed* and *invected*

0012-1606/00 $35.00

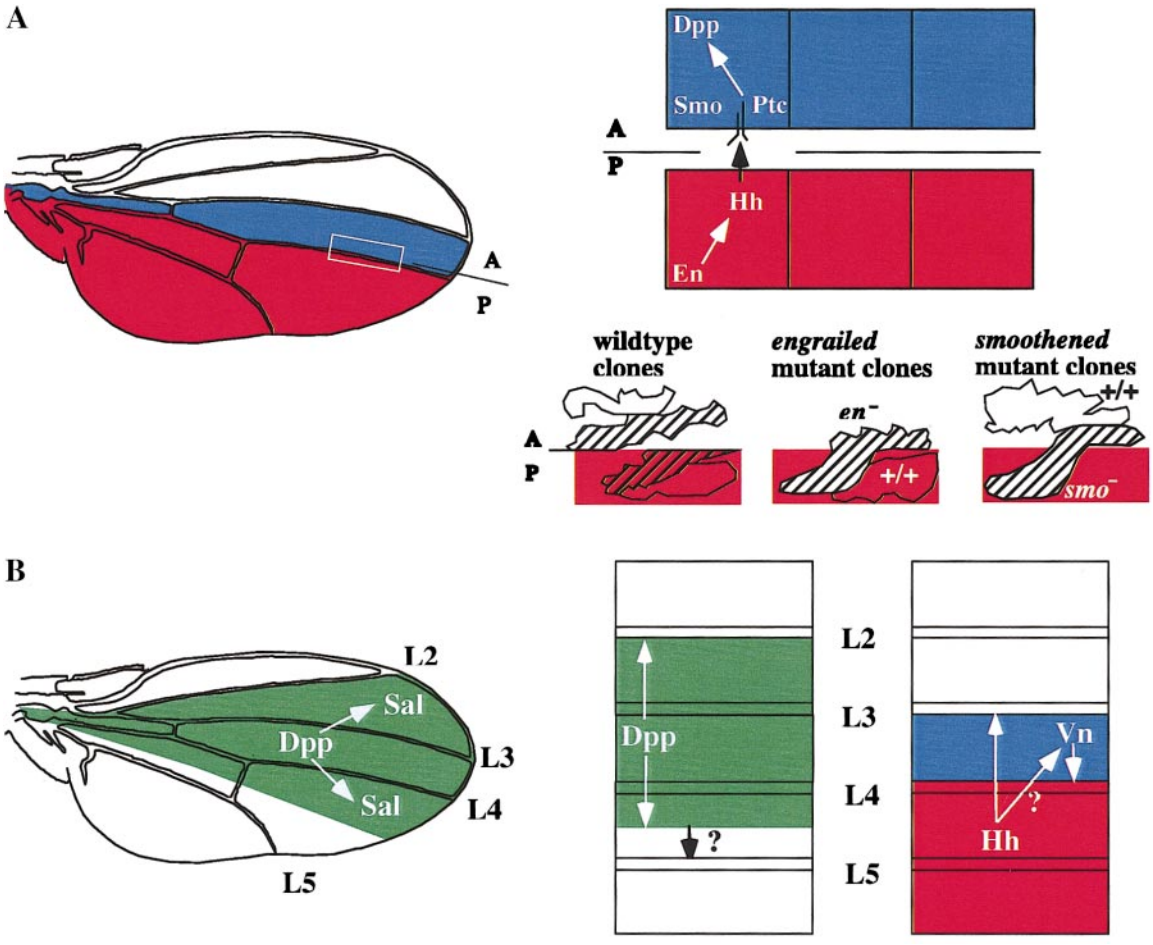

FIG. 1. Anteroposterior territorial subdivision of the wing primordium. (A) Posterior identity is conferred by the expression of the selector genes engrailed/invected (red). (Top right) Hedgehog (Hh) is expressed in P cells and signals to anterior cells via the transmembrane proteins Smoothened (Smo) and Patched (Ptc) to induce the expression of Dpp (blue) along the AP compartment boundary. (Bottom right) Wild-type clones do not cross the AP boundary. engrailed/invected mutant clones induced in the P compartment can cross the AP boundary. smoothened mutant clones induced in the A compartment can cross the AP boundary. (B) Dpp and Hh subdivide the wing pouch into vein/intervein regions. Dpp induces the expression of Spalt (green). The anterior boundary of Spalt expression positions vein L2. The posterior Spalt boundary lies between veins L4 and L5 and acts indirectly to position vein L5. Hh acts directly to position vein L3 and indirectly via the neuregulin-like protein Vein (Vn, blue) to position vein L4.

are unable to sense the cell lineage restriction that normally separates A and P compartments and can cross the compartment boundary freely (Zecca *et al.*, 1995; Tabata *et al.*, 1995; Hidalgo, 1994). The idea that differential cell affinity might be responsible for compartment segregation was suggested by differences in the shape of mutant clones. *engrailed/invected* mutant clones located in the P compartment have smooth borders, suggesting that they try to minimize contact with posterior cells. This contrasts with the irregular outlines of mutant clones in the A compartment or of control clones in either compartment (illustrated in Fig. 1A).

Recent studies have suggested that the segregation of A and P cells depends in part on communication between compartments (Blair and Ralston, 1997; Rodriguez and Basler, 1997). P cells express Hh and induce Hh-responsive genes in nearby A cells (Basler and Struhl, 1994; Tabata and Kornberg, 1994). Hh signals through a receptor complex involving Patched and Smoothened (Marigo *et al.*, 1996; Stone *et al.*, 1996; Alcedo *et al.*, 1996; van den Heuvel and Ingham, 1996; Chen and Struhl, 1996, 1998). Smoothened functions as the signal-transducing component of the receptor, so clones of cells mutant for *smoothened* are not able to receive the Hh signal. *smoothened* clones can cross the AP compartment boundary (Blair and Ralston, 1997; Rodriguez and Basler, 1997; see also Lawrence *et al.*, 1999). Thus, the segregation behavior of A and P cells appears to depend on Hh signaling. This idea is further supported by the obser-

vation that clones of A cells mutant for Patched or PKA, which activate the Hh pathway, round up, and minimize contact with other A cells (Phillips *et al.*, 1990; Tabata *et al.*, 1995; Pan and Rubin, 1995). It appears then that Hh signaling is likely to alter the expression of a cell surface protein (or proteins) in A cells that changes their interaction with P cells. The molecules responsible for the behavior have not yet been identified.

Secondary Subdivision: Hh and Dpp Subdivide the Wing Pouch into Vein/Intervein Territories

Wing veins are the principle morphological readout of the AP patterning system in the adult wing (Sturtevant and Bier, 1995; illustrated in Fig 1B). Specification of vein and intervein territories depends on the activity of both the Hh and Dpp signaling systems. Hh forms a short-range activity gradient in the A compartment that regulates the expression of a number of target genes, including Dpp (reviewed in Lawrence and Struhl, 1996; Brook *et al.*, 1996). Hh signaling has been shown to define the position of the third longitudinal vein (L3) (Mullor *et al.*, 1997; Strigini and Cohen, 1997). Experiments using a membrane-tethered, nondiffusible form of Hh have shown that size of the intervein region between veins three and four depends on the ability of Hh to form a short-range gradient in the A compartment (Strigini and Cohen, 1997).

Hh also appears to have an indirect role in positioning vein L4 in the posterior compartment. The gene *vein* encodes a neuregulin-like protein that is thought to serve as a ligand for the EGF receptor (Simcox *et al.*, 1996; Schnepp *et al.*, 1996). *vein* is expressed in late third instar in A cells adjacent to the AP boundary, presumably under Hh control (Fig. 1B). Clones of cells mutant for *vein* in the anterior compartment lead to loss of vein L4 in the adjacent posterior region, whereas large posterior clones have no effect on vein L4 (García-Bellido *et al.*, 1994). This indicates that anterior expression of Vein is required to specify vein L4 in the posterior compartment and suggests that Hh acts indirectly via Vein to specify vein L4 in posterior cells. Vein is also expressed more broadly at other times in wing development and is required to support growth of the wing disc during early larval stages and for the last cell divisions in pupal wing.

Dpp serves as a secondary signal to relay information from AP boundary. Dpp forms a long-range activity gradient that regulates gene expression in A and P compartments (Nellen *et al.*, 1996; Lecuit *et al.*, 1996). The transcription factor Spalt-major and its homologue Spalt-related (referred to collectively as Spalt) are targets of Dpp that have been implicated in vein patterning (Fig. 1B). Clonal analysis shows that vein L2 forms immediately adjacent to cells expressing Spalt in the A compartment (de Celis *et al.*, 1996b; Sturtevant *et al.*, 1997). This is reflected by localized expression of the transcription factors Knirps and Knirps-related in cells adjacent to the Spalt-expressing cells (Lunde *et al.*, 1998). Knirps and Knirps-related are required for

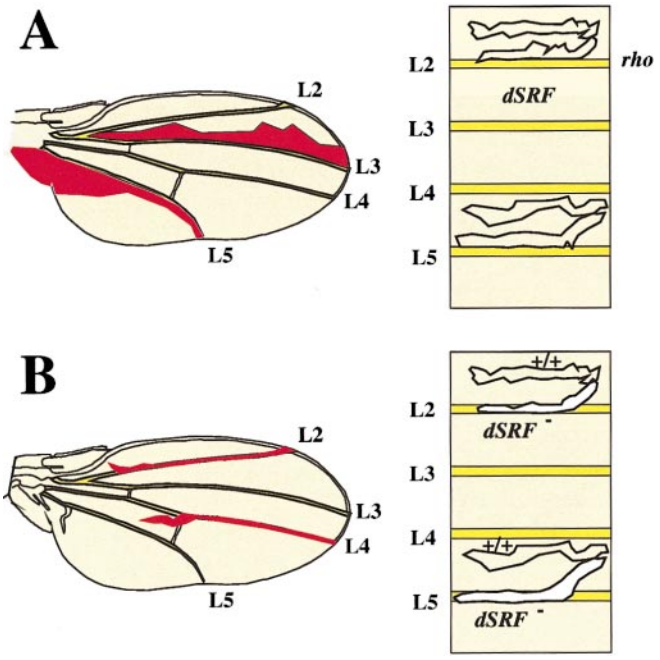

FIG. 2. Wing veins serve as boundaries. (A) Clones of genetically marked cells (red) tend to grow along wing veins without crossing them. Presumptive vein cells express rhomboid in the disc (rho, shown in dark yellow). Intervein cells express the *Drosophila* serum response factor in intervein cells(dSRF, shown in yellow). (B) dSRF mutant cells preferentially grow in the vein territory suggesting that differential affinities between vein and intervein cells help to maintain the vein/intervein subdivision.

Spalt-dependent formation of vein L2. The means by which the Spalt boundary leads to induction of Knirps in neighboring cells is not known.

The mechanism for positioning vein L5 is not understood, though there is some evidence that Spalt has an indirect effect. The posterior edge of the Spalt expression domain is located between veins L4 and L5. Yet, clones mutant for both genes affect the positioning of vein L5 even though the vein does not abut the Spalt-expression border (de Celis *et al.*, 1996b).

Do Wing Veins Delimit Units of Growth Control?

Several observations suggest that the presumptive veins may act as boundaries that influence the growth and mixing behavior of cells in the adjacent intervein territories. Analysis of genetically marked clones shows that cells tend to respect veins as boundaries to growth under normal conditions (González-Gaitán *et al.*, 1994). Clones of genetically marked cells tend to grow within a particular intervein territory. These clones can extend for considerable distances along a vein without crossing (illustrated in Fig. 2A). Marked clones tend to respect vein boundaries in wings bearing ectopic veins, such as *plexus* mutant wings, sug-

gesting that it is simply the presence of the vein that determines this cell behavior (Baonza and Garcia-Bellido, 1999). When given a growth advantage, clones of Minute$^+$ cells can cross veins freely, indicating that veins do not act as strict boundaries. Rather they appear to constrain cell behavior so as to delimit intervein territories as units in which growth occurs.

Vein and intervein territories are marked by differences in gene expression as the disc develops (illustrated in Fig. 2B). The *Drosophila* serum-response-factor homologue, Blistered, is expressed throughout the interveins and is required to allow cells to adopt intervein identity (Montagne *et al.*, 1996; Roch *et al.*, 1998). In *dSRF/blistered* mutant wings all cells adopt vein identity and the interveins are lost, suggesting that repression of blistered is required for vein differentiation. In this context the behavior of clones of *dSRF/blistered* mutant cells is interesting. Unlike wild-type clones which grow in intervein territories and which may run adjacent to veins without crossing them, *dSRF/blistered* mutant cells preferentially segregate into the vein even when given a growth advantage (Roch *et al.*, 1998). The borders between *blistered* mutant cells and wild-type cells is typically smooth, suggesting that vein and intervein cells may have a differential cell affinity.

Rhomboid is expressed in all presumptive vein territories where it is thought to activate Spitz, a ligand for the EGF receptor (Sturtevant *et al.*, 1993, 1997). In some viable combinations of *rhomboid* and *vein* alleles, all veins are missing and the wing is considerably reduced in size (García-Bellido *et al.*, 1994). Blistered is expressed throughout the wing in this situation, suggesting that Rhomboid-dependent activation of the EGF receptor is required to repress Blistered expression in presumptive veins (Roch *et al.*, 1998). The loss of veins in these genotypes can be suppressed by reducing Blistered activity, suggesting a regulatory loop between vein and intervein territories which depends in part on Rhomboid activity.

Other signaling systems have been implicated in this regulatory loop. The Notch-ligand, Delta, is expressed in presumptive veins. Delta regulates Notch expression in adjacent intervein cells and limits Rhomboid expression to the presumptive veins (de Celis and Bray, 1997). Argos, a secreted antagonist of EGF-receptor signaling, is also expressed in the presumptive veins and may help to regulate Delta expression (Sawamoto *et al.*, 1994; Schweitzer *et al.*, 1995; de Celis *et al.*, 1997). Thus a complex interplay between different signaling systems appears to be involved in distinguishing vein and intervein territories.

The behavior of vein and intervein cells is probably a consequence of these signaling events. The two populations generally do not mix, suggesting a difference in cell affinity. As a consequence of this affinity boundary, growth tends to take place within a given intervein territory. This raises the question of whether intervein territories might function as semiautonomous units of growth control.

PRIMARY AND SECONDARY SUBDIVISION OF THE LEG ALONG THE PROXIMAL–DISTAL AXIS

Primary Subdivision: Proximal and Distal Leg Domains Are Not Compartments

Primary subdivision of the leg along the PD axis does not involve the formation of compartment boundaries. No lineage restriction separates leg from body wall (Steiner, 1976; Wieschaus and Gehring, 1976; illustrated in Fig. 3). Wingless and Dpp form overlapping activity gradients that specify cell fates along the proximal–distal axis of the leg by regulating the expression of transcription factors that define several distinct domains (illustrated in Fig. 3A; Lecuit and Cohen, 1997). The disc is initially subdivided into proximal and distal domains by inducing Distal-less and repressing Homothorax and Teashirt expression (Diaz-Benjumea *et al.*, 1994; Abu-Shaar and Mann, 1998; González-Crespo *et al.*, 1998; Wu and Cohen, 1999). Homothorax (Hth) and Teashirt (Tsh) expression correspond to the presumptive body wall, coxa, and trochanter segments. Distal-less is transiently expressed in the primordia of all leg segments (Weigmann and Cohen, 1999) and is later restricted to the distal tibia and tarsal region and to a ring in the trochanter (Diaz-Benjumea *et al.*, 1994; Gorfinkiel *et al.*, 1997; Campbell and Tomlinson, 1998). Slightly later, Wg and Dpp induce Dachshund expression in a ring that includes the presumptive femur, tibia, and proximal tarsus (Lecuit and Cohen, 1997; Mardon *et al.*, 1994). Each of these genes is required for formation of specific regions of the leg, but their expression domains do not correspond precisely to the future segments of the adult leg.

The primary subdivision of the leg into proximal and distal domains does not define proximal and distal founder-cell populations that are kept separate thereafter (illustrated in Fig. 3B). Lineage-tracing experiments have shown that there is a significant net flow of cells from the proximal domain into the distal domain in normal development (Weigmann and Cohen, 1999). To cross between these domains cells must be able to change their pattern of gene expression. Proximal cells must lose Homothorax expression and be able to express Distal-less to cross into the distal leg (Fig. 3B; Cohen and Jürgens, 1989; Wu and Cohen, 1999). The outer ring of Dll expression seems to represent a transitional zone where cells change their identity from proximal to distal as the disc grows. Formation of this ring is important for formation of proximal leg segments. In the absence of *extradenticle* or *homothorax* activity the ring does not form and the separation between proximal leg segments and the body wall is lost with the result that femur and body wall structures fuse (Rauskolb *et al.*, 1995; Abu-Shaar and Mann, 1998; González-Crespo *et al.*, 1998; Wu and Cohen, 1999). Although there is no boundary of cell lineage restriction subdividing the PD axis into proximal and distal territories, separation of the territories is maintained by differential gene expression. At present the

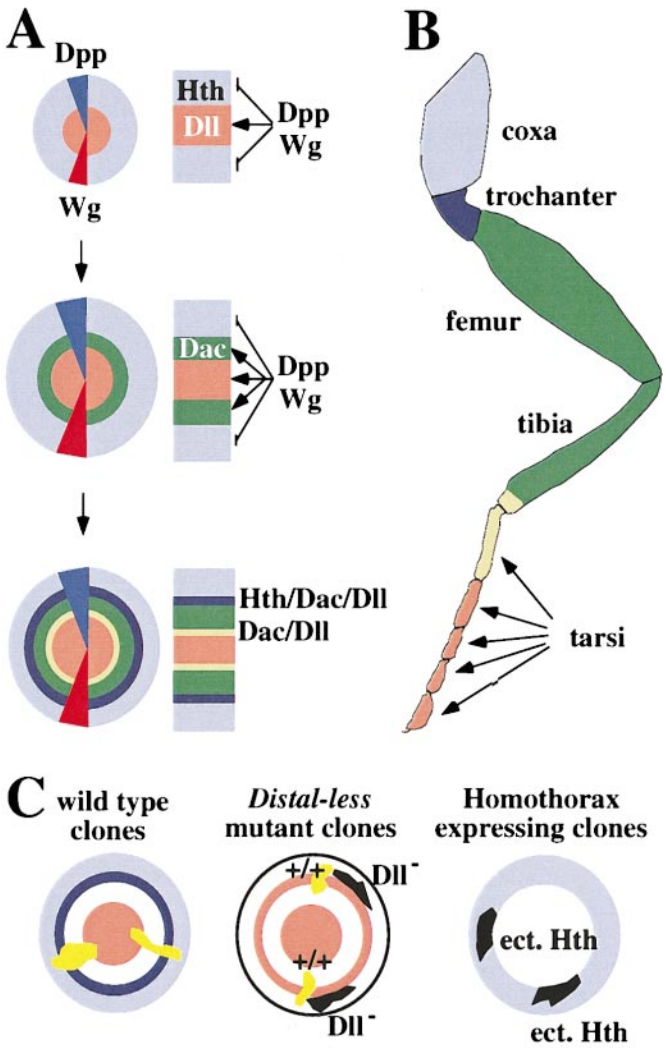

FIG. 3. Proximal–distal subdivision of the leg. (A) Stages of PD axis formation are depicted from top to bottom. Wg and Dpp are expressed in wedge shaped expression domains along the AP boundary of the leg disc (Dpp dark blue; Wg red). Wg and Dpp induce Dll (pink) and repress Homothorax (Hth, pale blue) to generate a primary subdivision into two territories. Wg and Dpp subsequently induce Dachshund (green) in a ring separating the Dll and Hth domains. Further refinement of the pattern leads to overlapping expression domains (bottom). The outer ring where Dll, Dachshund and Hth are coexpressed (dark blue) corresponds approximately to the trochanter and is needed to prevent leg segments from fusing with the body wall. (B) Projection of the expression domains onto the adult leg. The distal expression domains do not coincide with leg segments in a simple way. (C) Cell behavior at the Dll/Dac Hth ring. Clones of marked but otherwise wild-type cells freely cross the ring (+/+, yellow; at left). Distal-less mutant clones in the proximal domain cannot enter the ring (Dll^-, black; center). In distal regions these clones generally sort out from the epithelium and are lost (Wu and Cohen, 1999). Clones of cells forced to express Hth (right panel) can enter the ring but cannot cross into more distal territory. Cells must change their pattern of gene expression to cross the ring.

mechanism by which the cell populations are kept separate is not known. A mechanism based on differences in affinity between proximal and distal cells seems likely because clones of Hth-expressing cells produced in the distal domain sort out from the surrounding distal disc epithelium. Likewise clones of Dll-expressing cells sort out from the proximal Hth-expressing cells (Wu and Cohen, 1999).

Secondary Subdivision: Leg Segments

At later stages of leg development the disc is subdivided into presumptive segments. As noted above the segment borders do not align precisely in all cases with the domains of gene expression defined by Wg and Dpp along the PD axis of the leg (Fig. 3A). Segmentation is prefigured by the expression of a number of genes in ring patterns in the leg disc (Cohen, 1993). Among these are genes suggesting a role for the Notch pathway in leg segmentation (de Celis *et al.*, 1998; Bishop *et al.*, 1999; Rauskolb *et al.*, 1999). Reporter gene expression suggests that Notch signaling is activated in cells at the distal end of each forming segment, and clonal analysis shows that Notch pathway activity is required for segment boundary formation and its ectopic activation induces formation of extra joints (de Celis *et al.*, 1998; Bishop *et al.*, 1999; Rauskolb *et al.*, 1999). As in the case of the wing veins and wing margin, localized activation of the Notch pathway at the forming leg segment boundaries appears to involve a feedback loop leading to upregulation of the ligands Serrate and Delta in cells adjacent to those which have maximal Notch activity (de Celis and Bray, 1997; de Celis *et al.*, 1997; Micchelli *et al.*, 1997). Because of the topology of the leg, it is not known whether leg segments serve as boundaries to clone growth.

DO INTERVEIN TERRITORIES AND LEG SEGMENTS FUNCTION AS UNITS OF GROWTH CONTROL?

How do the long-range gradients of Wg and Dpp influence the size of the developing appendages? One model suggests that size and pattern are specified directly by the long-range ligand gradients (Lawrence and Struhl, 1996; Serrano and O'Farrell, 1997). According to this view growth stops when the gradient achieves a particular slope or local value, so that size would be defined with reference to a set of global positional cues. In the wing, the model would predict that the Dpp gradient directly determines growth along the AP axis and that the size of A and P compartments (illustrated in Fig. 4A). An alternative model is that the Dpp morphogen gradient defines a limited number of domains (e.g., Fig. 4B) and that subsequent patterning and size regulation is done with reference to these secondary subdivisions.

Two sets of observations may help to distinguish between these possibilities in the wing disc. First, clones of cells lacking *extramacrochaetae* (which encodes a helix–loop–helix protein) can eliminate an intervein territory and

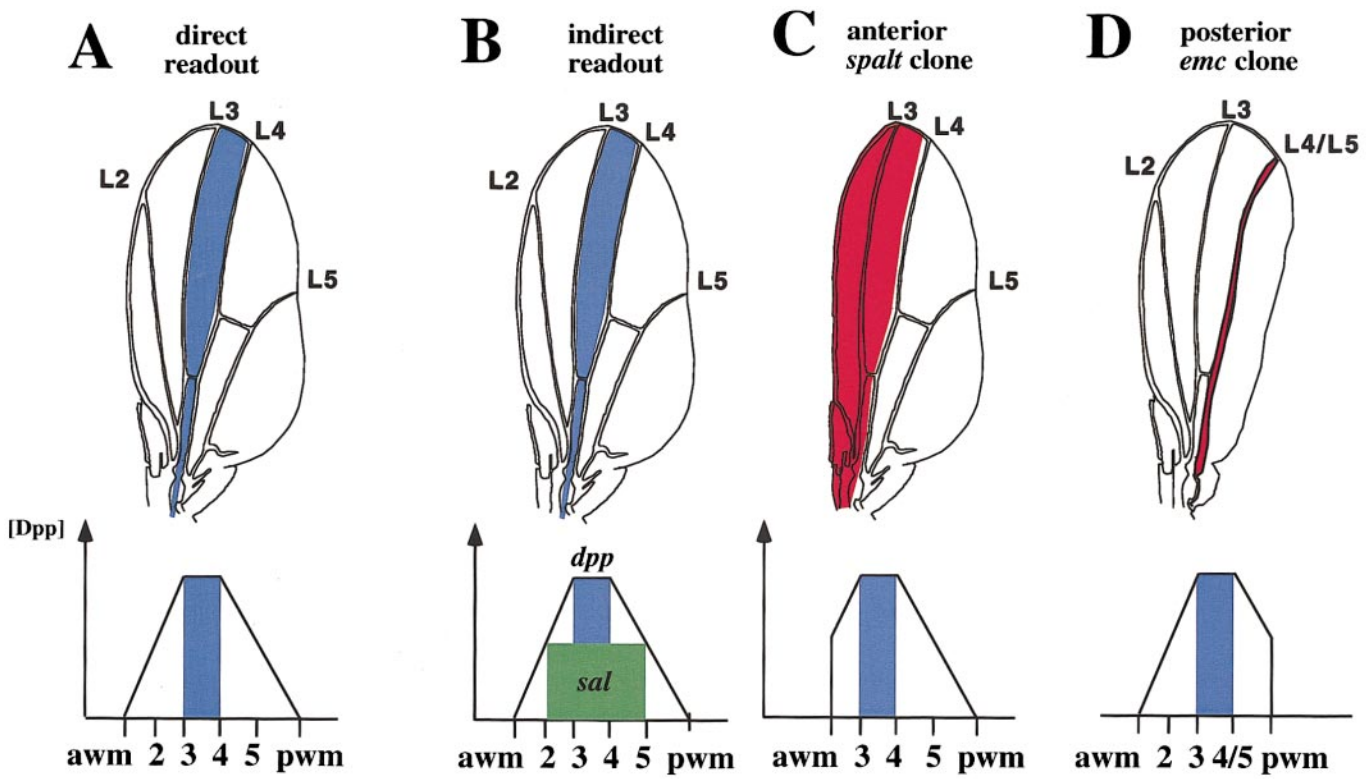

FIG. 4. Long-range vs local growth control. (A) Model for direct long-range influence of Dpp on growth and pattern along the AP axis of the wing. The slope and amplitude of the gradient would directly control the size of A and P compartments. (B) Model illustrating an indirect role for Dpp mediated through secondary subdivision of the axis. Different thresholds of Dpp induce the activation of the target genes. Spalt (green) has been implicated in specifying vein positions. (C) Depiction of a spalt mutant clone filling the A compartment (red). Such clones cause loss of vein L2. The A compartment is reduced to two intervein regions of approximately normal size. According to the gradient model the first vein (at the anterior margin) would be specified at a higher level in the Dpp gradient than in normal discs (B). Comparable effects are seen in the posterior compartment where the clones lead to loss of vein 5. (D) Depiction of an extramacrochaetae (emc) clone (red) causing loss of the region between veins 4 and 5 and fusion of the veins. The size of the region posterior to vein L5 is relatively normal, suggesting that posterior compartment size is not directly determined by the slope or amplitude of the Dpp gradient.

cause fusion of the flanking veins, without changing the size of the adjacent territories (de Celis *et al.*, 1996a; illustrated in Fig. 4D). In the example shown, the size of the region posterior to vein 4/5 is not increased to compensate for the missing intervein territory, as might be expected if the Dpp gradient directly controlled the size of the P compartment. This illustrates that removing an intervein territory has relatively little effect on the size of the adjacent territory as long as the vein is present. In contrast, a second set of experiments shows that removing a vein can have a significant impact on the size of the adjacent territories. Clones of cells mutant for Spalt can eliminate vein 2 (as illustrated in Fig. 4C). Loss of the vein causes the two adjacent "intervein" territories to be reduced to approximately the size of a single intervein territory (de Celis *et al.*, 1996b). In the absence of vein L2, the spacing between vein L3 and the anterior margin is similar to the normal spacing between vein L2 and the margin.

These observations are difficult to reconcile with a model in which the Dpp gradient directly controls final wing size along the AP axis. Rather, they suggest that specification of vein/intervein territories may be an important intermediate step in the patterning/growth regulation process. Careful studies of the pattern of cell division in *rhomboid* and *vein* mutants show that the reduced size of these wings is due to a decrease in the number of cell divisions in the pupal wing disc. The pupal cell divisions begin near the veins and progress in a wave-like pattern into intervein regions (García-Bellido *et al.*, 1994; Schubiger and Palka, 1987; Milan *et al.*, 1996). When veins are absent, as in these mutants, the late divisions do not occur. These observations suggest an important role for veins in controlling growth of intervein territories.

Can a comparison be drawn between wing vein/intervein territories and leg segments? In some sense, specification of leg segment boundaries might be considered comparable to the specification of vein–intervein boundaries. Both reflect the formation of reiterated patterns that subdivide a larger

developmental field. In the wing, a reasonably good case can be made that these territories function as semiautonomous units of size regulation at later stages of development. There is some evidence that disruption of segment boundaries in the leg can lead to reduced growth of the flanking segments (de Celis *et al.*, 1998; Bishop *et al.*, 1999; Rauskolb *et al.*, 1999); however, it remains to be determined whether leg segments function as autonomous units of size regulation. The similarities between these systems are intriguing.

HOW IS SIZE MEASURED?

Recent studies have suggested that overall size regulation in the fly depends on control of cell growth by the PI3 kinase pathway (Leevers *et al.*, 1996; Böhni *et al.*, 1999; Weinkove *et al.*, 1999). Other studies have shown that the patterning cues control tissue size or volume and that this can be uncoupled from control of cell number (Weigmann *et al.*, 1997; Neufeld *et al.*, 1998). In this review we have discussed evidence that patterning and growth control depend on subdivision of the limb field into smaller functional units. Cell communication within these units must play a central role in size regulation. One of the exciting challenges will be to determine how size is measured in these units and how this impacts upon activity of the signaling systems that control cell and tissue growth.

ACKNOWLEDGMENTS

We thank Michalis Averof, Maura Strigini, Birgit Kerber, and other colleagues in the laboratory for helpful discussions and comments on the manuscript. M.M. is a fellow of Human Frontiers Science Foundation.

REFERENCES

Abu-Shaar, M., and Mann, R. (1998). Generation of multiple antagonistic domains along the proximodistal axis during *Drosophila* leg development. *Development* **125,** 3821–3830.

Alcedo, J., Ayzenzon, M., von Ohlen, T., Noll, M., and Hooper, J. (1996). The *Drosophila smoothened* gene encodes a seven pass membrane protein, a putative receptor for the Hedgehog signal. *Cell* **86,** 221–232.

Baonza, A., and Garcia-Bellido, A. (1999). Dual role of *extramacrochaetae* in cell proliferation and cell differentiation during wing morphogenesis in *Drosophila. Mech. Dev.* **80,** 133–146.

Basler, K., and Struhl, G. (1994). Compartment boundaries and the control of *Drosophila* limb pattern by *hedgehog* protein. *Nature* **368,** 208–214.

Bishop, S. A., Klein, T., Martinez Arias, A., and Couso, J. P. (1999). Composite signalling from Serrate and Delta establishes leg segments in *Drosophila* through Notch. *Development* **126,** 2993–3003.

Blair, S. S. (1995). Compartments and appendage development in *Drosophila. BioEssays* **17,** 299–309.

Blair, S. S., and Ralston, A. (1997). Smoothened-mediated Hedgehog signalling is required for the maintenance of the anterior-posterior lineage restriction in the developing wing of *Drosophila. Development* **124,** 4053–4063.

Böhni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andruss, B. F., Beckingham, K., and Hafen, E. (1999). Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1-4. *Cell* **97,** 865–875.

Brook, W. J., and Cohen, S. M. (1996). Antagonistic interactions between Wingless and Decapentaplegic responsible for dorsal–ventral pattern in the *Drosophila* leg. *Science* **273,** 1373–1377.

Brook, W. J., Diaz-Benjumea, F. J., and Cohen, S. M. (1996). Organizing spatial pattern in limb development. *Annu. Rev. Cell Dev. Biol.* **12,** 161–180.

Campbell, G., and Tomlinson, A. (1998). The roles of homeobox genes *aristaless* and *Distal-less* in patterning legs and wings of *Drosophila. Development* **125,** 4483–4493.

Carroll, S. B. (1998). From pattern to gene, from gene to pattern. *Int. J. Dev. Biol.* **42,** 305–309.

Chen, Y., and Struhl, G. (1996). Dual roles for Patched in sequestering and transducing Hedgehog. *Cell* **87,** 553–563.

Chen, Y., and Struhl, G. (1998). In vivo evidence that Patched and Smoothened constitute distinct binding and transducing components of a Hedgehog receptor complex. *Development* **125,** 4943–4948.

Cohen, S. M. (1993). Imaginal disc development. *In* "*Drosophila* Development" (M. Bate and A. Martinez Arias, Eds.), pp. 747–841. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Cohen, S. M., and Jürgens, G. (1989). Proximal-distal pattern formation in *Drosophila:* Cell autonomous requirement for *Distal-less* gene activity in limb development. *EMBO J.* **8,** 2045–2055.

Crick, F. H., and Lawrence, P. A. (1975). Compartments and polyclones in insect development. *Science* **189,** 340–347.

de Celis, J. F., Baonza, A., and Garcia-Bellido, A. (1996a). Behaviour of *extramachrochaetae* mutant cells in the morphogenesis of the *Drosophila* wing. *Mech. Dev.* **53,** 209–221.

de Celis, J. F., Barrio, R., and Kafatos, F. C. (1996b). A gene complex acting downstream of *dpp* in *Drosophila* wing morphogenesis. *Nature* **381,** 421–424.

de Celis, J. F., and Bray, S. (1997). Feed-back mechanisms affecting Notch activation at the dorsoventral boundary in the *Drosophila* wing. *Development* **124,** 3241–3251.

de Celis, J. F., Bray, S., and Garcia-Bellido, A. (1997). Notch signalling regulates veinlet expression and establishes boundaries between veins and interveins in the *Drosophila* wing. *Development* **124,** 1919–1928.

de Celis, J. F., Tyler, D. M., de Celis, J., and Bray, S. (1998). Notch signaling mediates segmentation of the *Drosophila* leg. *Development* **125,** 4617–4626.

Diaz-Benjumea, F. J., Cohen, B., and Cohen, S. M. (1994). Cell interactions between compartments establishes the proximal–distal axis of *Drosophila* limbs. *Nature* **372,** 175–179.

Diaz-Benjumea, F. J., and Cohen, S. M. (1995). Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. *Development* **121,** 4215–4225.

García-Bellido, A. (1975). Genetic control of wing disc development in *Drosophila. In* "Cell Patterning: Ciba Foundation Symposium" (S. Brenner, Ed.), pp. 161–182. Associated Scientific Publishers.

García-Bellido, A., Cortés, F., and Milán, M. (1994). Cell interactions in the control of size in *Drosophila* wings. *Proc. Natl. Acad. Sci. USA* **91,** 10222–10226.

García-Bellido, A., Ripoll, P., and Morata, G. (1973). Developmental compartmentalisation of the wing disk of *Drosophila. Nature New Biol.* **245,** 251–253.

García-Bellido, A., Ripoll, P., and Morata, G. (1976). Developmental compartmentalization in the dorsal mesothoracic disc of *Drosophila. Dev. Biol.* **48,** 132–147.

González-Crespo, S., Abu-Shaar, M., Torres, M., Martinez, C., Mann, R. S., and Morata, G. (1998). Antagonism between extradenticle function and hedgehog signalling in the developing limb. *Nature* **394,** 196–200.

González-Gaitán, M., Paz Capdevila, M., and García-Bellido, A. (1994). Cell proliferation patterns in the wing imaginal disc of *Drosophila. Mech. Dev.* **40,** 183–200.

Gorfinkiel, N., Morata, G., and Guerrero, I. (1997). The homeobox gene Distal-less induces ventral appendage development in *Drosophila. Genes Dev.* **11,** 2259–2271.

Hidalgo, A. (1994). Three distinct roles for the *engrailed* gene in *Drosophila* wing development. *Curr. Biol.* **4,** 1087–1098.

Jiang, J., and Struhl, G. (1996). Complementary and mutually exclusive activities of Decapentaplegic and Wingless organize axial pattern during *Drosophila* limb development. *Cell* **86,** 401–409.

Lawrence, P. A., and Struhl, G. (1996). Morphogens, compartments and pattern: Lessons from *Drosophila*? *Cell* **85,** 951–961.

Lawrence, P. A., Casal, J., and Struhl, G. (1999). The Hedgehog morphogen and gradients of cell affinity in the abdomen of *Drosophila. Development* **126,** 2441–2449.

Lecuit, T., Brook, W. J., Ng, M., Calleja, M., Sun, H., and Cohen, S. M. (1996). Two distinct mechanisms for long-range patterning by Decapentaplegic in the *Drosophila* wing. *Nature* **381,** 387–393.

Lecuit, T., and Cohen, S. M. (1997). Proximal–distal axis formation in the *Drosophila* leg. *Nature* **388,** 139–145.

Leevers, S. J., Weinkove, D., MacDougall, L. K., Hafen, E., and Waterfield, M. D. (1996). The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth. *EMBO J.* **15,** 6584–6594.

Lunde, K., Biehs, B., Nauber, U., and Bier, E. (1998). The knirps and knirps-related genes organize development of the second wing vein in *Drosophila. Development* **125,** 4145–4154.

Mardon, G., Solomon, N. M., and Rubin, G. M. (1994). *dachshund* encodes a nuclear protein required for normal eye and leg development in *Drosophila. Development* **120,** 3473–3486.

Marigo, V., Scott, M. P., Johnson, R. L., Goodrich, L. V., and Tabin, C. J. (1996). Conservation of *hedgehog* signaling: Induction of a chicken *patched* homolog by *sonic hedgehog* in the developing limb. *Development* **122,** 1225–1233.

Micchelli, C. A., Rulifson, E. J., and Blair, S. S. (1997). The function and regulation of cut expression on the wing margin of *Drosophila:* Notch, Wingless and a dominant negative role for Delta and Serrate. *Development* **124,** 1485–1495.

Milan, M., Campuzano, S., and Garcia-Bellído, A. (1996). Cell cycling and patterned cell proliferation in the *Drosophila* wing during metamorphosis. *Proc. Natl. Acad. Sci. USA* **93,** 11687–11692.

Montagne, J., Groppe, J., Guillemin, K., Krasnow, M., Gehring, W., and Affolter, M. (1996). The *Drosophila* serum response factor gene is required for the formation of intervein tissue of the wing and is allelic to *blistered. Development* **122,** 2589–2597.

Mullor, J. L., Calleja, M., Capdevila, J., and Guerrero, I. (1997). Hedgehog activity, independent of Decapentaplegic, participates in wing disc patterning. *Development* **124,** 1227–1237.

Nellen, D., Burke, R., Struhl, G., and Basler, K. (1996). Direct and long-range action of a Dpp morphogen gradient. *Cell* **85,** 357–368.

Neufeld, T. P., de la Cruz, A. F., Johnston, L. A., Edgar, B. A. (1998). Coordination of growth and cell division in the *Drosophila* wing. *Cell* **93,** 1183–1193.

Neumann, C. J., and Cohen, S. M. (1997a). Long-range action of Wingless organizes the dorsal–ventral axis of the *Drosophila* wing. *Development* **124,** 871–880.

Neumann, C. J., and Cohen, S. M. (1997b). Morphogens and pattern formation. *BioEssays* **19,** 721–729.

Pan, D. J., and Rubin, G. (1995). Protein kinase A and hedgehog act antagonistically in regulating *decapentaplegic* transcription in *Drosophila* imaginal discs. *Cell* **80,** 543–552.

Phillips, R. G., Roberts, I. J. H., Ingham, P. W., and Whittle, J. R. S. (1990). The *Drosophila* segment polarity gene patched is involved in a position-signalling mechanism in imaginal discs. *Development* **110,** 105–114.

Rauskolb, C., Smith, K. M., Peifer, M., and Wieschaus, E. (1995). *extradenticle* determines segment identities throughout *Drosophila* development. *Development* **121,** 3663–3673.

Rauskolb, C., and Irvine, K. D. (1999). Notch-mediated segmentation and growth control of the *Drosophila* leg. *Dev. Biol.* **210,** 339–350.

Roch, F., Baonzo, A., Martin-Blanco, E., Garcia-Bellido, A. (1998). Genetic interactions and cell behaviour in blistered mutants during proliferation and differentiation of the *Drosophila* wing. *Development* **125,** 1823–1832.

Rodriguez, I., and Basler, K. (1997). Control of compartmental affinity boundaries by hedgehog. *Nature* **389,** 614–618.

Sawamoto, K., Okano, H., Kobayakawa, Y., Hayashi, S., Mikoshiba, K., and Tanimura, T. (1994). The function of argos in regulating cell fate decisions during *Drosophila* eye and wing vein development. *Dev. Biol.* **164,** 267–276.

Schnepp, B., Grumbling, G., Donaldson, T., and Simcox, A. (1996). Vein is a novel component in the *Drosophila* epidermal growth factor receptor pathway with similarity to the neuregulins. *Genes Dev.* **10,** 2302–2313.

Schubiger, M., and Palka, J. (1987). Changing spatial patterns of DNA replication in the developing wing of *Drosophila. Dev. Biol.* **123,** 145–153.

Schweitzer, R., Howes, R., Smith, R., Shilo, B. Z., and Freeman, M. (1995). Inhibition of *Drosophila* EGF receptor activation by the secreted protein Argos. *Nature* **376,** 699–702.

Serrano, N., O'Farrell, P. (1997). Limb morphogenesis: connections between patterning and growth. *Current Biol.* **7,** 186–195.

Simcox, A. A., Grumbling, G., Schnepp, B., Bennington-Mathias, C., Hersperger, E., and Shearn, A. (1996). Molecular, phenotypic, and expression analysis of vein, a gene required for growth of the *Drosophila* wing disc. *Dev. Biol.* **177,** 475–489.

Steiner, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila* melanogaster. *Wilhelm Roux's Arch.* **180,** 9–30.

Stone, D. M., Hynes, M., Armanini, M., Swanson, T. A., Gu, Q., Johnson, R. L., Scott, M. P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J. E., de Sauvage, F., and Rosenthal, A. (1996). The tumor-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog. *Nature* **384,** 129–134.

Strigini, M., and Cohen, S. M. (1997). A Hedgehog activity gradient contributes to AP axial patterning of the *Drosophila* wing. *Development* **124,** 4697–4705.

Sturtevant, M. A., Biehs, B., Marin, E., and Bier, E. A. (1997). The spalt gene links the A/P compartment boundary to a linear adult structure in the *Drosophila* wing. *Development* **124,** 21–32.

Sturtevant, M. A., and Bier, E. (1995). Analysis of the genetic hierarchy guiding wing vein development in *Drosophila*. *Development* **121,** 785–801.

Sturtevant, M. A., Roark, M., and Bier, E. (1993). The *Drosophila rhomboid* gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signalling pathway. *Genes Dev.* **7,** 961–973.

Tabata, T., and Kornberg, T. (1994). Hedgehog is a signalling protein with a key role in patterning *Drosophila* imaginal discs. *Cell* **76,** 89–102.

Tabata, T., Schwartz, C., Gustavson, E., Ali, Z., and Kornberg, T. B. (1995). Creating a *Drosophila* wing de novo: The role of *engrailed* and the compartment border hypothesis. *Development* **121,** 3359–3369.

van den Heuvel, M., and Ingham, P. W. (1996). *smoothened* encodes a receptor-like serpentine protein required for *Hedgehog* signaling. *Nature* **382,** 547–551.

Weigmann, K., and Cohen, S. M. (1999). Lineage tracing cells born in different domains along the PD axis of the developing *Drosophila* leg. *Development* **126,** 3823–3830.

Weigmann, K., Cohen, S. M., Lehrer, C. F. (1997). Cell cycle progression, growth, and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* CDR-kinase. *Development* **124,** 3555–3563.

Weinkove, D., Neufeld, T. P., Twardzik, T., Waterfield, M. D., Leevers, S. J. (1999). Regulation of imaginal disc cell size, cell number, and organ size by *Drosophila* Class 1A phospoinositide 3-kinase and its adaptor. *Curr. Biol.* **9,** 1019–1029.

Wieschaus, E., and Gehring, W. (1976). Clonal analysis of primordial disc cells in the early embryo of *Drosophila melanogaster*. *Dev. Biol.* **50,** 249–263.

Wu, J., and Cohen, S. M. (1999). Proximal distal axis formation in the *Drosophila* leg: Primary subdivision into proximal and distal domains by Homothorax, Teashirt and Distal-less expression. *Development* **126,** 109–117.

Zecca, M., Basler, K., and Struhl, G. (1995). Sequential organizing activities of *engrailed*, *hedgehog* and *decapentaplegic* in the *Drosophila* wing. *Development* **121,** 2265–2278.

Zecca, M., Basler, K., and Struhl, G. (1996). Direct and long-range action of a Wingless morphogen gradient. *Cell* **87,** 833–844.

Received for publication June 7, 1999
Revised August 6, 1999
Accepted September 11, 1999